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**Toxicological Review of Formaldehyde Inhalation; Draft  
Toxicological Review of Formaldehyde in Support of Summary  
Information on the Integrated Risk Information System; Notice of  
Availability and Request for Comment 87 Fed. Reg. 72 (Apr. 14,  
2022) Docket ID No. EPA-HQ-ORD-2010-0396**

Date June 9, 2022

Dear Dr. Cascio,

USEPA identifies seven issues with the BBDR modeling (Maintext, Table 2-24, p 2-70) and state that 3 of these *"have major impacts on qualitative and quantitative conclusions drawn from the modeling"* (p. 2-69, line 21). Retaining the numbering used in Table 2-24, these issues are:

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3	Uncertainties and variability in the rat cell labeling data, the derivation of cell division rates from these data, and their applicability to human cell division rates.
6	Sensitivity of model results to the use of historical control animals drawn from all NTP cancer bioassays.
7	Uncertainties in assumed division and death rates of initiated cells.

In the following, I address these three concerns about the BBDR modeling and its use to support risk assessment. First, however, I provide general comments on USEPA's approach to the evaluation of the BBDR modeling and the conclusion by USEPA that the modeling is too uncertain to support risk assessment.

**General Comments**

The overall formaldehyde dataset for F344 rat nasal tumors and related mechanistic information is probably the most extensive such dataset in existence for any chemical. Given that the USEPA's own Guidelines for Carcinogen Risk Assessment (USEPA 2005) clearly state a default preference for data-driven risk assessment, its multi-decade effort to develop a cancer risk assessment for formaldehyde, and its own significant resources in computational toxicology and risk assessment, it is curious that USEPA has not developed a BBDR model for formaldehyde. USEPA IRIS has, however, devoted considerable resources (Crump

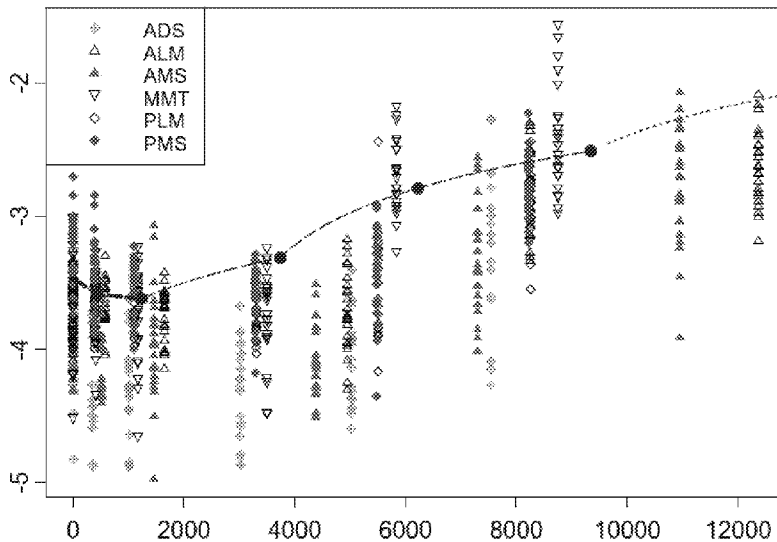
et al. 2008; Subramaniam et al. 2007; Subramaniam et al. 2008) to evaluate the CIIT BBDR model (Conolly et al. 2003; Conolly et al. 2004). Those of us who have developed biologically motivated models in support of risk assessment understand that no model is perfect, and that model analysis and iterative refinement support reductions in model uncertainty. It would have been interesting and informative, and consistent with their own Cancer Guidelines, if USEPA IRIS had chosen to use their analyses to develop a refined version of the CIIT model. Instead, USEPA has rejected the CIIT BBDR model and turned to more empirical approaches. In making this choice, USEPA is effectively saying that relevant mechanistic data are confusing and increase rather than decrease uncertainty, a position that is inconsistent with the 2005 guidelines. In this regard, it is worth noting that I was employed in USEPA/ORD from 2005 through my retirement in February 2000 but was not consulted by IRIS on their evaluation of the CIIT BBDR model.

A full examination of the uncertainties associated with empirical modeling of dose-response, side-by-side with the uncertainties that USEPA has identified in the CIIT BBDR modeling, would be informative. For example, one could ask about the relative uncertainty of dosimetry based on CFD modeling in anatomically realistic models of rat and human nasal passages versus empirical modeling of dose-response that does not specifically address dosimetry at all. Ignoring dosimetry does not, and should not, remove concern for the role of dosimetry as a determinant of dose-response. Uncertainty about dosimetry exists for any model of toxicant/carcinogen dose-response. Is a dose-response analysis that explicitly describes dosimetry inherently more uncertain than an approach that wholly ignores dosimetry? This same argument, comparing data-driven and empirical descriptions, can be applied to each of the main components of the BBDR model. Empirical models effectively hide explicit uncertainties about the shapes of the dose-response curve because the empirical models do not acknowledge the mechanistic determinants of dose-response. The CIIT BBDR model, on the other hand, explicitly describes these determinants. This situation is reminiscent of the early days of PBPK modeling (1980's – 1990's) where PBPK models were sometimes said to increase uncertainty in the characterization of pharmacokinetics relative to empirical PK models. The need here, both for PBPK models and for the BBDR modeling for formaldehyde, is to step back and ask what determines the behavior of interest and to then realize that only relevant data and its inclusion into the dose-response analysis can address these uncertainties. BBDR modeling is simply a tool for integrating data on the mechanistic determinants of dose-response to provide a capability for predicting the entire dose-time response surface.

The following consideration of explicit uncertainties in the BBDR for issues 3, 7 and 7 will address whether or not the BBDR modeling is of sufficient quality that it avoids introducing uncertainty due to technical insufficiency.

### Issue 3 - Labelling index data

Figure B-18 in the Appendices shows the effect of inhaled formaldehyde on the rate of cell division in the rat nose as described by Monticello et al. (1991) and Monticello et al. (1996):



**Figure B-18. Logarithm of normal cell replication rate  $\alpha_w$  versus formaldehyde flux (in units of pmol/mm<sup>2</sup>-hr) for the F344 rat nasal epithelium.**

This dataset, which is from the 2<sup>nd</sup> CIIT bioassay (Monticello et al. 1991, 1996) consists of 240 datapoints collected from 5 sites in the rat nose, at 8 timepoints (0.14, 0.57, 1.29, 6.0, 12, 26, 52 and 78 weeks) and at 6 exposure levels (0, 0.7, 2, 6, 10 and 15 ppm). While USEPA also refers to several other labeling index datasets (Figure 2-5, Maintext, p. 2-66), none of those other datasets approach the CIIT bioassay dataset in terms of coverage of dose-response, time-course, and of multiple nasal sampling sites. As can be seen in Figure B-18 above, there is considerable variation in labeling at any given flux value. This is not surprising for those who have worked in the laboratory collecting such data. Notwithstanding the variability in the data, a clear J-shape is apparent, with an initial decrease in labeling with increasing flux to a nadir in the labeling index slightly below a flux of 2,000 pmol/(mm<sup>2</sup>-hr). At higher flux values, which are associated with cytolethal effects of inhaled formaldehyde, the labeling index increases, reflecting regenerative cellular proliferation subsequent to cell killing. Heck and Casanova (1999) suggested that DNA-protein crosslinks (DPX) due to inhaled formaldehyde act as physical blocks to the progression of the replication complex along the DNA strand, and thereby effectively reduced the rate of cell replication. This would be analogous to a car sideways in the road, blocking traffic. The theory proposed by Heck and Casanova (1999) is supported in more recent literature (e.g. Nakamura and Nakamura 2020).

The J-shaped dose-response for labeling index has significant implications for formaldehyde risk assessment. In the CIIT BBDR model, formaldehyde exerts a low dose linear directly mutagenic effect as a function of the formation of DPX and, simultaneously, the J-shaped dose-response for the rate of cell division. The human version of the CIIT BBDR model (Conolly et al. 2004) predicted a J-shaped dose-response for tumor risk, with risk only increasing above background at about 2 ppm inhaled formaldehyde. This result was, of course, dramatically different from USEPA's assessments, which

identify inhalation unit risks in the range of a few ppb. In this context, USEPA makes much of the variability in the labeling index data (see Time variability of labeling data, Appendices, p. B-54). The variability is used to justify alternate dose-responses that do not include the J-shape, including dose-responses that are monotonically increasing in cell division rate as a function of flux/inhaled ppm (e.g. Figures B-24 & B-25, Appendices, p. B-63). These alternative dose-responses can dramatically change the risks predicted by the BBDR model. Given the size and consistency of the Monticello et al. (1991, 1996) dataset, these alternative dose-response exercises are less than convincing. Yes, if the data were different, the model behavior would be different! I suggest that USEPA should provide a clearly stated justification for use of cell replication dose-responses that are not J-shaped. If the size and quality of the labeling index data described by Monticello et al. (1991, 1996) is not to be trusted, then what is?

Conolly et al. (2003) developed an expression for labeling index as a function of flux. In so doing they averaged data across sampling sites and calculated time weighted averages (TWA) across the various sampling time points. This one-dimensional condensation of the data preserved the J-shape seen in Figure B-18 and was shown by Gaylor et al. (2004) to describe a statistically significant departure from monotonic dose-response. USEPA state that "*TWA of cell labeling data over sites was found to be problematic on multiple accounts*" (Maintext, p. 2-71, lines 23-24). I agree that calculation of division rates as a function of both time (age of rat) and flux is preferable. In fact, this two-dimensional approach is being used in an ongoing update of the BBDR model that is not otherwise considered here. However, USEPA is using this and similar criticisms of the CIIT BBDR modeling of the labeling index data to justify use of alternative, much more empirical dose-response models that do not capture our understanding of the biological processes involved. As noted above, the one-dimensional expression for division rate as a function of flux retains the main qualitative feature of the labeling index dataset, its statistically significant J-shape. Does USEPA really think that an empirical dose-response function that completely ignores the CIIT bioassay labeling index dataset provides greater certainty in the prediction of human risk?

Labeling index data for bioassay exposure durations from 1 day through 6 weeks (Monticello et al. 1991) were measured by injecting BrdU while subsequent time points (Monticello et al. 1996) used osmotic minipumps implanted for 3 days. Calculation of a division rate constant, which is needed as an input to the clonal growth model, from labeling index data, requires an estimate of the length of the interval over which tissue is exposed to the labeling agent. Since this estimation is problematical for injection studies, the injection data were first transformed to equivalent minipump data using a factor of 6.83, which was the ratio of minipump labeling index to injection labeling index for all the control data. This factor was calculated from a total of 40 data points, 20 from pulse labeling and 20 from pump labeling. Conolly et al. (2003) then used a formula for the conversion of labeling index data into division rate constants due to Moolgavkar and Luebeck (1992). USEPA correctly note that that pulse label data should not be used with this formula. However, they apparently fail to understand that the pulse label data were transformed into equivalent pump data before they were used for calculation of division rate constants (see Maintext p. 2-71 and Appendices, p. B-54, Uncertainty due to combining pulse and continuous labeled data). Thus, the USEPA statements that the use of the Moolgavkar and Luebeck (1992) formula is "extremely uncertain" (Maintext, p. 2-71, line 26) and "problematic" (Appendices, p. B-54, line 17) and is itself problematical.

## Issue 6 - Use of historical controls

The CIIT BBDR model combined the historical controls from corn oil gavage and inhalation bioassays and found 13 nasal squamous cell carcinomas. Subramaniam et al. (2008) pointed out that 12 of these

tumors were from corn oil gavage bioassays and only 1 was from an inhalation bioassay. It is possible that corn oil gavage can occasionally lead to reflux of corn oil into the nasal passages, leading to irritation, which is a risk factor for tumor development (Dr. Kevin Morgan, personal communication). Thus, rejection of the corn oil gavage control tumors is appropriate.

In the current draft IRIS assessment, USEPA states (Formaldehyde Appendices, p. B-50, lines 29-38):

*A crucial point needs to be noted with regard to the use of inhalation NTP historical controls (i.e. cases B and E) in the two-stage clonal growth modeling. The single relevant tumor in the NTP inhalation studies came from the very first NTP inhalation study, dated 1976, and the animals in this study were from Hazelton Laboratories, whereas the concurrent animals were all from Charles River Laboratories. Similar problems arise with inclusion of several other NTP inhalation studies. As mentioned before, genetic and other time-related variation can lead to different tumor and survival rates, and in general it is recommended that use of historical controls be restricted to the same kind of bioassays and to studies within a 5–7 year span of the concurrent animals (Peddada et al. 2007). Thus, it is problematic to assume that the tumor in the 1976 NTP study is representative of the risk of SCCs in the formaldehyde bioassays.*

The most straightforward way to address this concern is to assume that nasal SCC do not occur spontaneously in the F344 rat. This would mean that no control nasal SCC would be seen in a concurrent control group of any size. However, as noted by USEPA, the lack of control tumors poses a problem for the CIIT BBDR model (Appendices, p. B-49, lines 10-12:

*...the extrapolation to human risk by using the approach in Conolly et al. (2004) becomes particularly problematic when only concurrent controls are used, because then the mutational contribution to formaldehyde-induced risk in humans becomes unbounded.*

The problem arises in the scale-up of the rat BBDR model to the human version. This scale-up involves the ratio:

$$\frac{\mu_{N_{basal_h}}}{\mu_{N_{basal_r}}}$$

where  $\mu_{N_{basal_h}}$  is the probability of mutation per cell division (pmuth) for controls in the human version of the model and  $\mu_{N_{basal_r}}$  is the equivalent for rats (pmutr). If there are no rat control tumors, then the rat probability is 0 and the ratio is undefined.

When the CIIT BBDR model was developed, we worked with 13 control tumors and did not anticipate the possibility of there being no control tumors. Is it reasonable to use this issue to justify rejection of the CIIT model? USEPA describes numerous alternative versions of the CIIT model in the IRIS Maintext and Appendices and in the supporting publications (Crump et al. 2008; Subramaniam et al. 2007; Subramaniam et al. 2008). Thus, the ability within USEPA to develop alternative versions of the BBDR is not a constraint. Is there a reasonable alternative for scale-up of the CIIT rat BBDR model to the human version that USEPA could have implemented?

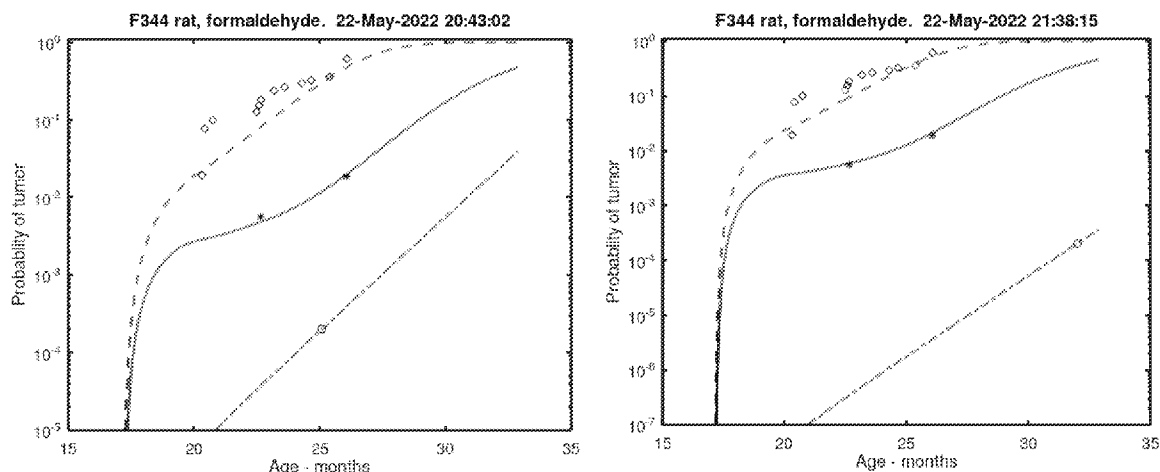
Peto's paradox (Caulin and Maley 2011) tells us that cancer risk does not correlate with cell number. If risk did correlate with cell number, species that are larger and longer-lived than rats (humans, whales, etc.) would have correspondingly larger risks of cancer, which is not the case. Thus, pmuth is smaller than pmutr. The ratio of human to rat basal mutation rates described above and used in Conolly et al. (2004) is a means of accounting for Peto's paradox in the scale-up of the BBDR model from the rat to

human. While that approach cannot be used when there are no control tumors, alternative methods of scaling are available. For example:

1. Set up a two-stage clonal growth (MVK) model for the rat nose as in Conolly et al. (2003).
2. Without specifying exposure to formaldehyde or any other chemical, adjust pmutr to produce a small lifetime tumor incidence, say  $10^{-6}$ . Repeat with increasing values of pmutr until lifetime tumor incidence is 100%. This exercise will need to draw on relevant literature for biologically reasonable values of the growth advantage for intermediate cells (cells with one mutation) relative to normal cells (e.g. Conolly and Kimbell 1994).
3. Set up the MVK model for the human respiratory tract as in Conolly et al. (2004).
4. As in 2 above, adjust pmuth to obtain end of life tumor risks from  $10^{-6}$  to 1.0 (100%).
5. The ratio of pmuth to pmutr at given levels of lifetime risk will describe rat to human scaling of the MVK model. The values of pmuth will be several orders of magnitude smaller than the corresponding values of pmutr.
6. This scaling would then be used with the human BBDR model to evaluate cancer risks associated with exposure to formaldehyde. For example, the calculated scaling factor(s) would be used to adjust the value of KMUrat, which defines the relationship between DPX and pmutr, to obtain KMUhuman.

This is an example of how USEPA IRIS could have scaled the rat BBDR model to the human and used the human BBDR model in support of risk assessment, specifically for the case of no control tumors. So doing would have shown a commitment to data-driven risk assessment and alignment of this new IRIS assessment with the 2005 Cancer guidelines.

It is possible that the probability of spontaneous nasal SCC in F344 rats is not zero but is sufficiently small to explain the absence of control tumors in the concurrent controls and in the larger NIH inhalation control cohort (if the single observed control SCC is discounted). A straightforward modeling technique can be used to visually evaluate possible values of the control probability of tumor when no such tumors have been observed:



The panel on the left shows a single control tumor at age 20 months, which is the actual age at which the single inhalation control tumor occurred. The panel on the right shows the control tumor moved out to about 33 months. This shifting of the control tumor to a later age reduces the probability of tumor at

earlier ages. In the panel on the right, the probability of tumor at 25 months is about 2 orders of magnitude smaller than in the panel on the left. However, it's worth noting that, in the panel of the left, control risk at 33 months is almost 1 in 10, which is unrealistically high. Thus, even though the simulation does a good job of describing the observed control tumor at 25 months, the overall simulation is clearly inaccurate. This result suggests that assuming that there are no relevant control tumors may be more appropriate.

## Issue 7 – Initiated cells

In the 2-stage clonal growth (MVK) model, a major component of the CIIT BBDR model, normal (N) cells mutate into initiated (I) cells and mutation of an initiated cell creates a tumor cell. After a time delay, a single tumor cell expands clonally to become a clinically detectable squamous cell carcinomas (SCC). In the CIIT BBDR model, characterization of the N cell population is data-based. The number of N cells at risk of neoplastic transformation, and the division and death rates of these cells are all well informed by data (Conolly et. al. 2003). The incidence of SCC is also well characterized, in this case by both their dose-response and time course behaviors (see the 2 upper curves in the panels above, which show tumors at 6 and 10 ppm). However, essentially no data usable for development of the BBDR model are available for I cells. The fact that both the N cell population and tumor incidence are well characterized highly constrains the possible values of the parameters that determine the growth and mutation kinetics of I cells. Parametrization of I cells is described in detail in Conolly et al. (2003). Accurate descriptions of the dose-response and time course of the nasal SCC were obtained using maximum likelihood optimization of the I cell parameter values. USEPA (see Maintext, p. 2-73, Kinetics of initiated cells) and Crump et al. (2008) perturb the values of these optimized parameters, particularly the I cell division rate, and show that BBDR model predictions of tumor incidence changes quite dramatically. This is, however, expected behavior when the optimized value of a sensitive parameter is arbitrarily changed. USEPA states:

*...extremely small differences in assumptions for  $\alpha I$  (the division rate of I cells) appear to have extremely large effects on the human model predictions (Maintext, 2-75, lines 1-2)*

and

*Such an extreme sensitivity indicates that the formaldehyde human TSCE model is unstable in response to the slight perturbations carried out to the assumed values of  $\alpha I$ , and is therefore not robust. (Maintext, p. 2-75, lines 7-9).*

These conclusions by USEPA are incorrect because they ignore the significance of the optimized value of the division rate constant for I cells. An indication of why USEPA is making this error is provided by the following:

*There are currently no data of any kind, even in rats, to inform the effect of formaldehyde on the kinetics of initiated cells. (Maintext, p. 2-75, lines 15-16)*

USEPA appears to not understand that the richness of the datasets for N cells and SCC highly constrains the kinetics of I cells, and specifically the value of the I cell division rate constant. It is just plain wrong to say that there are no relevant data for I cells.

## Summary & Conclusions

The current IRIS draft demonstrates a limited understanding of the CIIT BBDR model and, frankly, what seems to be a greater interest in discrediting the model than in refining/using it to support a data-



driven risk assessment for formaldehyde, as the USEPA's own Cancer Guidelines state should be the default approach for data-rich assessments.

USEPA IRIS is strongly encouraged to consider the uncertainties of the BBDR model relative to the hidden uncertainties embedded in the empirical dose-response functions that they are more comfortable with. As previously noted, lack of explicit description of mechanism (i.e. toxicokinetics & MOA) in a dose-response function does not avoid accountability for the implications toxicokinetics and MOA in assessing the dose-response and its consequent implications for the risk assessment, especially when so much relevant data are available.

The specific issues addressed in the sections above are only a subset of a full characterization of USEPA's evaluation of the CIIT BBDR model. It is hoped that they nevertheless provide a useful perspective.

Yours sincerely

A handwritten signature in black ink that reads "Rory B. Conolly". The signature is fluid and cursive, with a horizontal line underneath the name.

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